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Mosaic Disease of Tobacco

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MOSAIC DISEASE OF TOBACCO

By

George H. Chapman.

Thesis submitted for the degree of Doctor of Philosophy.

Massachusetts Agricultural College

Amherst, Mass.

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INTRODUCTION.

The observations and conclusions reported in the following pages are the results of several years of more or less continuous investigation on the part of the writer, and deal with the causes, occurrence, appearance and the probable methods of control of this well known disease of the tobacco and related plants. Enough has been accomplished so that it is believed wise to add still another paper to the already long list of literature which has been published on this disease. During the time in which these experiments have been in progress much new literature has appeared dealing with this subject, some of which has helped the writer by verifying his results and bringing out new facts concerning the disease; but on the other hand some of the work appears to have been done in a hasty manner and possibly erroneous conclusions are drawn in some cases, thus adding to the already large amount of confusing subject matter which has to do with this disease. The experiments carried on by the writer were begun in a general way in 1907 and have been repeated several times during the years subsequent to that date, new lines of investigation both in the field and laboratory having been added as occasion demanded. Some considerable time has been spent in verifying the results obtained by other recent inves-

tigators, and an attempt has been made to gather together in a broad, general way, as well as in detail, all the reliable information possible about this interesting disease, as well as to bring out new facts in regard to it. More attention has been given to the biochemical aspects of the problem than has heretofore been done by investigators.

HISTORICAL SUMMARY.

In the following paragraphs is given a brief resumé of the more important work done on the mosaic disease of tobacco up to the present time, and as an excellent critical review of the literature, etc. up to 1902 is given by A. F. Woods in his work on the subject, the same is

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Woods, A. F. Observations on the Mosaic Disease of Tobacco. Bul. Bur. Plant Ind., U.S.D.A., #18 (1902).

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quoted in full below. He states:-- "Adolph Mayer was the first to make a careful study of the trouble. He demonstrated that it could not be caused by an insufficient supply of mineral nutrients.

Mayer, Adolph. Uber die Mosaikkrankheit des Tabaks. Landw. Versuchsstation 32: 451--467 (1886). Review of the same article in Journ. of Mycology 7: 382--385 (1894).

He found as much nitrogen, potassium salts, phosphates. calcium and magnesium present in the soils and plants where the disease

occurred as in the soils where the disease did not occur. He also found that the trouble was apparently distributed over the field without regard to the soil conditions.

Since tobacco requires much lime, liming the soil was tried, but the disease was not prevented thereby. Mayer further kept hotbeds in some cases rather moist, in others dry, and then again richly or poorly manured with nitrogen; but in no case could he determine that the conditions in question caused the disease. He also found that variations in the temperature of the hotbeds apparently had no effect; neither did crowding, which produced partial etiolation, appear to have any effect on the disease. Seeds from flowers in which self-fertilization was prevented he found to be just as susceptible to the disease as seeds produced without such precautions, but on soil on which the disease had once appeared it was again produced. According to his observations also, the trouble was not often found on soil used for the first time for tobacco. He further proved that the juice of the diseased leaves injected with the juice of healthy plants did not develop the disease. He was not able to produce it by injecting diseased juice into other solanaceous plants. Where the diseased juice was injected into tobacco the same trouble developed in from ten to eleven days. Heating to 60° C. did not destroy the infectious substance; at 65° to 75° it was attenuated, and at 80° it was killed.

After Mayer had shown the absence of animal and fungous parasites he supposed bacteria to be the cause of the disease,

but all his efforts with bacteria cultivated from the surface of diseased leaves, and also with different mixtures of bacteria, failed to produce it. Nevertheless he thought that there must be certain pathogenic bacteria present in those soils in which the disease appeared, and therefore proposed to change the soil in the hotbeds and to devote the fields where tobacco had been cultivated to other crops. He also recommended the use of mineral rather than organic manures.

These general results were confirmed by several subsequent investigators, Not, however, till Beijerinck took hold of the question,

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Beijerinck, M. W. Verhandelingen der Koninklijke Akademie van Wetenschappen te Amsterdam. Dee 16: #5. -- See also Centb. f. Bakt. Par. etc. II:5:27-33 (1899).

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was much of importance added to our knowledge of the malady. He proved the absence of bacteria in the development of the disease. He showed that the juice of the plant filtered through Chamberland filters, while remaining perfectly clear and free from bacteria, still retained the power of infection. A small drop of it injected hypodermically into the growing bud was sufficient to give the plant the disease. He found that only dividing (meristematic) cells can become diseased. Diseased tissue kept its infectious qualities even after drying, and retained its injurious properties in the soil during the winter. Weak solutions of formalin did not kill the virus, but heating

to boiling point did. Fresh, unfiltered juice was more effective than an equal amount of filtered juice. He found that soil around diseased plants may infect the roots of healthy plants, but he did not determine whether direct transference is possible through healthy root surfaces, or whether insects, by injuring the roots, favored infection. He defines the milder form of the disease as a suffering of the chlorophyll bodies. Later a general disease of the plasmatic contents of the cells sets in.

In field conditions as a final stage the swollen green areas become marked with small dead spots, but these did not appear on plants grown under glass. Under certain conditions he observed that plants apparently recover from the disease; i.e., the new growth appeared to recover. He found that the infective material, whatever it might be, could be transported through considerable distances in the plant, but could cause the disease only in the dividing cells. He assumed the virus to be a non-corpuscular, fluid-like material, which had the power of growth when in contact, in a sort of symbiotic way, with the growing cells-- "a living fluid contagium."

Shortly after Beijerinck's paper, Sturgis published a

Sturgis, W. A. Mosaic Disease of Tobacco. Conn. Agr. Exp. Sta. Rept. 250-254 (1898).

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critical review of the work done on the disease up to that time, with numerous valuable results and observations made in Connecticut, where the trouble is known as "calico" or "mottled top."

The results obtained by Sturgis and observations made by him on tobacco in Connecticut bore out the statements of other careful and critical workers, and greatly cleared up the field for further investigation. He came to the conclusion that on close, clayey soils the disease may be more abundant than on an open, porous soil. The disease is not contagious, but he could not state definitely as to its infectiousness; it is not caused by fungi, nematodes or parasitic insects, and the facts observed by him were not favorable to the theory of bacterial origin. He also came to the conclusion that the disease is not inherent in the seed, and looked upon it as a purely physiological trouble brought about by sudden interruptions of the normal plant metabolism. Koning, in his work,

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Koning, C. J. Die Flecken oder Mosaikkrankheit des hollandischen Tabaks. Zeitschrift für Pflanzenkr. 9:65-80.

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verified much of the work of Beijerinck and Mayer, and Woods

Woods, A. F. Inhibiting Action of Oxidase on Diastase. Science n.s. #262:17-19.

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later verified the work of these investigators and pointed out that in the diseased leaves there was an excess or excessive activity on the part of an enzyme belonging to the oxidases, and that the power of oxidation in the cells was inversely proportional to the amount of chlorophyll present, using the color as a basis of comparison. He also pointed out that there was a marked structural difference between the cells of the dark green

and light green areas, and proved to his own satisfaction that the light green areas are the truly diseased portions, a fact that will be referred to later in this paper. In a later careful investigation of the disease Woods arrived at the following conclusions, which were a great stride forward in our understanding of some phases of this baffling disease. He states:--

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Woods, A. F. l.c., p.2.

"The disease is not due to parasites of any kind, but is the result of defective nutrition of the young dividing and rapidly growing cells, due to a lack of elaborated nitrogenous reserve food accompanied by an abnormal increase in the activity of oxidizing enzymes in the diseased cells. The unusual activity of the enzyme prevents the proper elaboration of the reserve food, so that a plant once diseased seldom recovers. On the decay of the roots, leaves and stems of both healthy and diseased plants, the enzyme in question is liberated and remains active in the soil. The enzyme is very soluble in water and appears to pass readily through plant membranes. If the young plants take it up in sufficient quantity to reach the terminal bud, they become diseased in the characteristic way. Under field conditions there is little danger from infection in this manner, but in the seed bed the danger is much greater on account of the greater susceptibility of the young plants to the disease, and the greater amount of free oxidizing enzymes likely to be in the soil due to the decay of the roots and plants. New or steam sterilized soil should therefore be used for the seed bed.

I have shown that transplanting, especially when the roots are injured, may produce the disease. Great care must, therefore, be taken not to injure the roots in this process or in the subsequent cultivation, or to check the growth of the plants.

There is evidence that rapid growth, caused by too much nitrogenous manure or too high a temperature, is favorable to the disease. Why this should be the case has not been determined. It is probably connected with the manufacture of reserve nitrogen by the cells and its distribution to the rapidly growing parts.

Plants grown under such conditions are less able to stand successfully marked variations in temperature and moister conditions of soil and atmosphere. Variations of this kind favor the development of the disease in the less resistant plants.

Close, clayey soils, packing hard after rains and requiring constant tillage, are not favorable to the even growth of either the tops or roots of tobacco plants. In moist, cloudy weather the plants will grow too fast, and in hot, dry weather the soil is likely to bake, checking growth and making probable injury to the roots in cultivation. Such soils are very favorable to the development of the mosaic disease, as pointed out by Thaxter. He found that loosening the soil by liming and

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Thaxter, Conn. Agr. Exp. Sta. Rept. III:253 (1899).

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giving partial shade, thus causing a more even condition of growth, very greatly reduced the disease.

Crops grown under cheesecloth covers protected at the side are said to be remarkably free from the disease. The plants

make a steady, rapid growth, much greater than in ordinary field culture.

The disease is not, so far as observed, produced by a lack of soil nutrients, though from its nature we would expect that a deficiency of nitrogen, phosphoric acid, lime and magnesia might favor its development. Koning says that manuring with

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Koning, C. J. l. c., p.5.

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kainit and Thomas slag diminishes the extent of the disease. Mayer, Beijerinck and other investigators, however, agree that the trouble is not caused by the lack of any soil nutrients. It appears so far as my own investigations go that the trouble cannot be cured by giving the plants additional food of any kind. Over-feeding with nitrogen favors the development of the disease, and there is some evidence that excess of nitrates in the cells may cause an excessive development of the ferments that cause the disease. Very slight attacks of the disease known as "mottled top" are said not to injure the quality of the leaf to a sufficient extent to be noticeable commercially, though they may be less elastic and have a poorer burn and aroma than healthy leaves."

Hunger, in his work on the mosaic of Deli tobacco, verified

Hunger, F. W. T. De Mozaiek-ziekte bij deli Tabak. Med. s'Lands Plantentium, Batavaia. Deel 1:63 (1903).

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much of the work of previous investigators, and later on care-

fully planned and executed experiments proved that the disease

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----- Die Verbreitung der Mosaikkrankheit infolge der Behandlung des Tabaks. Centralbl. f. Bakt. Par. etc., II:11:405-408 (1908).

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was not contagious but was highly infectious, and believed that it could be carried from diseased to healthy leaves simply by touching, especially in the case of the young leaves, a fact that makes it necessary for the workman to use great care when looking for the tobacco bud worms, etc. in the buds. He was of the opinion that a rupture of the leaf was not necessary to induce the mosaic disease in plants.

Selby a year later showed this to be apparently true for

Selby, A. D. Tobacco Diseases. Ohio Agr. Exp. Sta. Bul. 15:88-95 (1904).

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tobacco grown in Ohio, and Hunger's statements were in his opinion in all respects confirmed. He also reported that "Blossoms of various plants were inoculated through the nectar by transmission of nectar from diseased plants, as by insect visitation. A slender brush of horse hair was used for this purpose. No evidences of the disease were observed as a result of this method."

Clinton was able to produce the trouble on tomatoes by inoculating with juice from a diseased tobacco plant and from the tomato so infected was able to reproduce the disease on the tobacco again by inocculation from the tomato, again showing the infectious nature of the disease, and that the troubles on

Clinton, G. P. Notes on Fungous Diseases, Etc. Conn. Agr. Exp. Sta. Rept. 1907-1908:857-858.

the tomato and tobacco were practically identical. This has been repeatedly verified by the writer and many other investigators.

Jensen, in his work on the disease, came to the conclusion

Jensen, H. Uber die Bekampfung der Mosaikkrankheit der Tabakpflanze. Centralbl. f. Bakt. Par. Etc. II. 15:440-445 (1906).

that the right way to get at the methods of control of the disease was by experimentation to obtain a resistant strain of tobacco, no matter what the cause of the disease might be, and he carried on some experiments along these lines. As yet no definite results have been reported by the investigators, but the time has probably been too short to obtain results along these lines.

Lodewijks stated that he was able to treat diseased plants

Lodewijks, T. A. Jr. Zur Mosaikkrankheit des Tabaks. Rec. Trav. bot. Neerlandais VII (1910).

by growing them in different colored lights, and in some cases bring about a cure. He states:-- "The mosaic disease cannot be diminished or stopped in its propagation by diminished light intensity. Neither diffused nor colored light has any noticeable effect on the disease, if the healthy leaves are not able to assimilate in normal daylight. Under the latter condition, however, diffused light exerts a retardation, red light dimin-

ishes the trouble, and blue light effects a cure. All the results may then be explained by the hypothesis that the virus formation diminishes with the intensity of the light, while in the healthy leaves, through the action of the virus, an anti-virus is formed, the action of which destroys the virus (immunity and antitoxin formation in the case of animals.)

Normally in the metabolism of the tobacco plant a substance is formed, the action of which is opposed to that of the equally normally occurring virus of mosaic disease, perhaps because it binds itself chemically to the latter.

Both substances, virus and anti-virus, may be increased by external factors or conditions. In the first case the plants become diseased with the mosaic disease; in the latter an immunity against the disease is brought about. Diminuation, decrease and cureoccur if the virus formation ceases or stops, and at the same time the formation of an anti-virus is taking place normally or is increased."

Translation from abstract of Lodewijks' paper in Bot. Centralbl. 114-518 (1910).

A discussion of Lodewijks' work is to be found later in this paper.

Allard in a recent work on the disease states that from

Allard, H. A. Mosaic Disease of Tobacco. U. S. D. A., Bur. Plant Ind. Bul. 40 (1914).

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the results of his experiments he is of the opinion that the trouble is not primarily physiological but it is parasitic in nature, but he is unable to throw any light on the nature of the parasite, and in spite of the conclusions drawn by him, none of his results so far as the writer as well as others interested in the problem are able to judge, has in any way weakened the theory that the trouble may be physiological in nature, and some of his results from the writer's point of view, seem to substantiate this idea of a physiological agency. Two points of great interest are brought out by him, viz., the mosaic as affecting the color of the corolla by blotching, etc., and the carrying of the disease by certain aphids. These points have not been noted before. In the following pages some of these points will be taken up in so far as they seem to bear out or refute the work done by the writer.

It may be seen from the foregoing resumé that the theory that the disease is physiological in nature is pretty generally accepted but the question of the exact causes bringing about these symptoms varies with different investigators. The writer's experiments are discussed in the following pages.

DESCRIPTION OF THE MOSAIC DISEASE ON TOBACCO.

Descriptions of the mosaic disease have been repeatedly given and it is so well known that there is little need of repetition at this point, but as a brief resumé of the salient characteristics of the disease will be given so that no misunderstanding may arise, as several other leaf troubles more or less chlorotic in character have often been confounded with the true

mosaic. The disease may show on the leaves at all stages of the growth from the seedling to the mature plant. It is often difficult in seedlings to diagnose the trouble definitely as the slight mottling and curl of the leaves may be due to other factors. As a rule in young plants the leaf is rougher and a permanent mottling is observed, very slight in character, however, and not to be confounded with the mottling due to normal metabolic processes which occurs under certain conditions of growth. As the disease progresses, however, the leaf is found to be divided into light and dark green areas; in mild cases there does not appear to be any marked leaf distortion, and the light green areas sometimes verge on the yellow in color. The dark green areas apparently deepen in color with the intensity of the disease, and in extreme cases the leaf is much distorted and the dark portions appear blister-like, due to their more rapid growth. The leaves, as a rule, are much stiffer and thicker to the feel than are the normal healthy leaves. Sometimes in the later stages of the disease there are found dry, dead, brown patches or spots on the leaves, usually where the dark green areas were originally, but in some cases the light green portions also show this extreme condidion. Both the light and dark areas show abnormalities in structure, nevertheless the light green areas are the more truly diseased ones, the dark green areas presenting different characteristics, and although showing changes in cell arrangement, etc., function more normally in many respects. Most investigators have held that the light green areas are the diseased portions of a leaf, but some have been of the opinion that the dark green areas

were the diseased portions. As will be seen from the writer's experiments the former is the more correct view as the increase in color intensity and the blistering of the dark green areas is due to the necessarily increased functioning thrown on these portions of the leaf.

Occasionally a leaf may be distorted in such a manner as to present the appearance of being little more than a long filament consisting principally of midrib, with but very little leaf surface. This condition has been observed by the writer in some instances, but should not be confounded with a similar trouble occurring on tobacco in certain regions which is of an unknown character, but which is not the true mosaic as it is not infectious. This trouble has been noted particularly in Java, etc., as is reported by Peters in his work on the diseases of tobacco. It has not been observed in tobacco fields in this region by the writer.

Peters, L. Krankheiten und Beschadigung des Tabaks. Mitteil. aus der Kaiser. Anstalt F. Land - u. Forstwirtschaft. Heft 13:64 (1912).

It is thought that soil and moisture conditions are responsible at least partially for this disease.

PLATE I.

Tobacco Mosaic.

(Various leaf malformation)

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Tobacco Mosaic.

(Various leaf malformation)

PATHOLOGICAL ANATOMY.

Leaves. As might be supposed, there are great differences in structure between normal, healthy leaves and leaves affected with the mosaic disease. These differences are greatest, naturally, in badly diseased leaves. Woods was the first to point out this fact, and his statements have been repeatedly verified by the writer.

Woods, A. F. Inhibiting Action of Oxidase on Diastase. Science, N. S., XI, No. 262:17-19 (1900).

He stated that the light colored areas were not normal and that "this difference consists in the fact that in badly diseased plants the palisade parenchyma of the light colored areas is not developed at all. All the tissue between the upper and lower epidermis consists of a spongy or respiratory parenchyma rather more closely packed than normal. In moderately diseased plants the palisade parenchyma of the light area is greatly modified. Normally the palisade parenchyma cells of a healthy plant are from four to six times as long as broad. In a moderately diseased plant, however, the cells are nearly as broad as they are long, or at most not more than twice as long as broad. As a rule the modified cells of the leaf pass abruptly into the normal cells of the green area."

From the above it can be seen that Woods was of the opinion that the light green areas were abnormal or diseased, and that the dark green areas were normal and healthy. The writer in

his observations found this to be true in general, but occasionally the dark green areas showed a more closely packed parenchyma than the normal leaf tissue, but always the palisade layer was well developed and approached the normal in character. The development or non-development of the palisade layer, as Woods hinted, is dependent on the degree of severity of the disease. The lighter the attack the less are the palisade cells and parenchyma tissue altered, and vice-versa. This the writer found to be true in so far as anatomical differences were concerned, but as will be noted later, the dark green, apparently normal healthy tissue contained some of the infective agent of the disease.

The structure of the dark green areas varies only slightly from that of the normal leaf, with the few exceptions above noted, and may be considered normal in character. The writer has sectioned many leaves in all stages of disease and these structural differences have always been found to occur in the manner above indicated. These differences in structure have been taken up more or less in detail as some investigators have held, and still hold, that the dark green areas are the part diseased, and that the light green areas are the normal, inasmuch as they approach the normal leaf in color in many cases, most probably basing their assumption on the fact that the dark areas form blister-like growths and are sometimes darker in color than normal leaves; but no one appears to have investigated the structure of the dark and light areas carefully in the case of the tobacco, except Woods. It was to verify Woods' statements that

the writer took this phase of the matter up, and mention will again be made of it in connection with the biochemistry of the leaf. There can be no doubt as to the correctness of Woods' contention that the light green areas are abnormal and diseased, but that the dark green areas are not diseased in certain cases cannot be so definitely stated. Their structure may be slightly modified by the increased functioning thrown on the healthy cells. On the other hand, it is fallacious to state that the light green are the healthy, and the dark green are the diseased portions of a leaf.

Plates I, II, and III show three cross sections from leaves, (I) being the cross section of a healthy leaf, (II) that of a light green area of a badly diseased leaf, and (III) the same of a dark green blister on the same leaf. It will be noted that the palisade layer is practically suppressed in (II) or the light green portion, while in (III) the palisade layer approaches the normal(I) in character except for a closer packing of cells in general. Milder cases of diseased leaves vary between these limits. These figures are from camera lucida drawings of material killed and fixed in medium chromacetic acid. In the material used the normal leaf section is somewhat thicker than those of the diseased leaf, but for comparative purposes is perfectly satisfactory.

Stem. The anatomical differences in the leaves of healthy and diseased tobacco plants have been given in the preceding paragraphs, and as it was desired to carry the investigations further to cover the entire plant, repeated examinations were made of both cross and longisections of stems of plants

in various stages of disease, and also of healthy, normal plants grown both in the field and greenhouse. It should be stated at this point that occasionally the writer has observed on the stems of some badly mosaiced plants a mottling, or rather, a streaking of the stem, a portion of which would be darker green than the remainder of the stem and this is without question a manifestation of the mosaic disease. Sections of such stems, however, showed absolutely no variation from those of normal plants, and in no case, although the examinations covered an extended period of time, was it possible to show any structural differences between the stems of badly diseased mosaic plants and those of healthy plants of the same age. Examinations of the stem close to the terminal apex of the plant revealed the same conditions as those made of other parts of the stem. No differences were observable except in the matter of size of cells, such as would naturally be expected when we take into consideration the difference in size and development of the stem near the terminal apex and the base of the plant.

Roots. In the same manner, roots of healthy and mosaiced plants were examined from time to time under all conditions of growth and severity of disease, and in every case the root structure was found to be normal. Root tips carefully killed and fixed in chromacetic acid showed no structural differences. It might be anticipated that as the disease manifests itself in the growing portions of the leaves exposed to the action of sunlight there might be a supplementary differentiation, so to speak, of tissue at the growing point of the root to function co-ordinately

with that of the aerial part of the plant. No such a condition was observable, however, and so far as the writer has been able to find, there is no such manifestation of local cell disturbances in the root such as are found in the leaf tissue.

The active principle of the disease, however, as has previously been noted, is without question present in all parts of the plants, and therefore it cannot be stated that it is confined to those parts which show structural variation.

FUNGI AND THE MOSAIC DISEASE.

Almost from the first it has been established that no fungi are associated with the cause and development of the mosaic disease of tobacco. In no case where careful work has been conducted under conditions eliminating the possibility of accidental infection has any fungus been found associated with the trouble. Cultures of fungi obtained occasionally from leaves have always been traceable to careless manipulation or external infection, and the fungus obtained failed to infect healthy plants, no matter what methods of inoculation were used.

The writer has occasionally obtained cultures on various media such as oat agar, tobacco leaf agar and prune agar from the tissue of the so-called "rusted" spots which are sometimes a late manifestation of the last stages of the mosaic, but like previous investigators, it was impossible to infect healthy plants from these cultures either by needle pricks, spraying or insetting the fungus mycelium into incisions in the leaf or stem.

PLATE II.

Section Through Normal Tobacco Leaf.

(a) Upper epidermis; (b) palisade layer; (c) parenchyma.

(Copy: traced from original drawing)

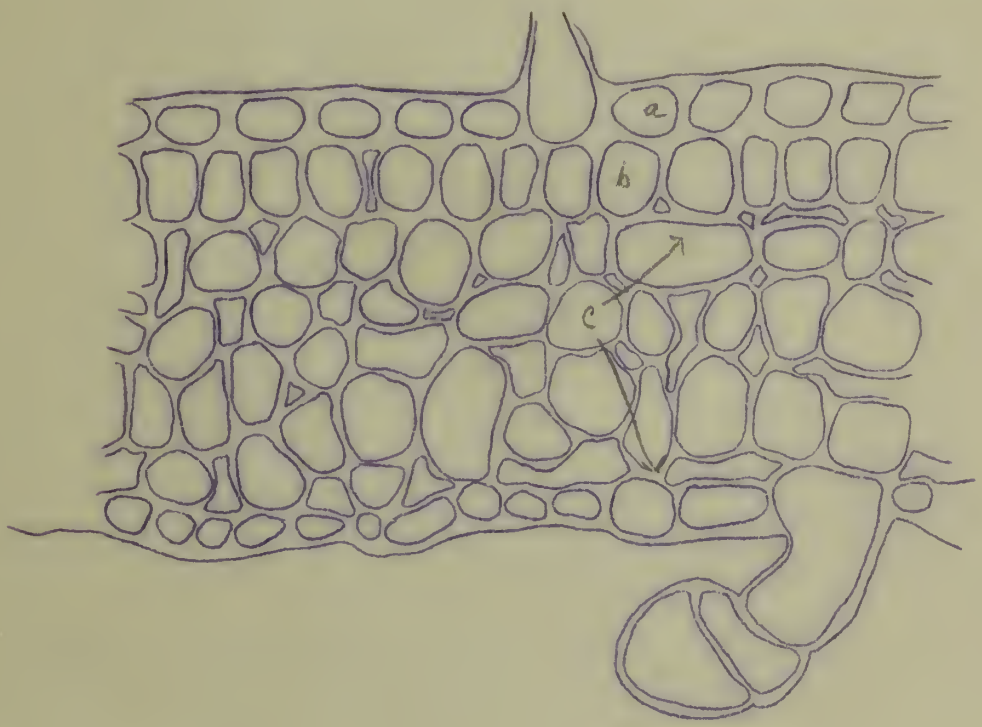

PLATE III.

Section Through Mosaic Diseased Tobacco Leaf.

(light green area)

(a) Upper epidermis; (b) Palisade layer; (c) Parenchyma.

(Copy: traced from original drawing)

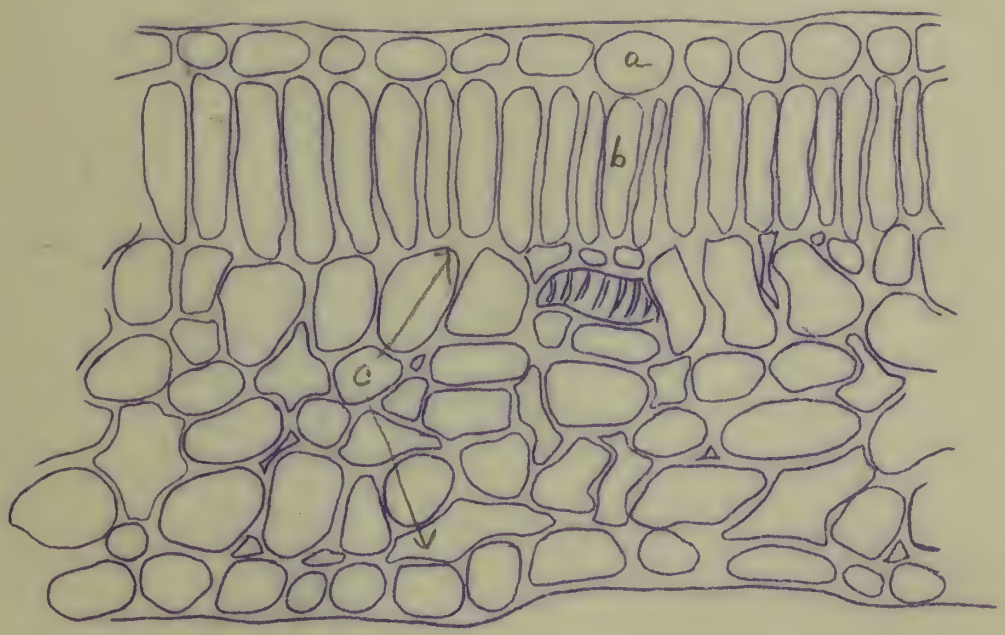

PLATE IV.

Section Through Mosaic Diseased Tobacco Leaf.

(dark green area)

(a) Upper epidermis; (b) Palisade layer; (c) Parenchyma.

(Copy: traced from original drawing.)

These experiments with fungi were made merely to demonstrate to the writer's own satisfaction that they could not be the causative agents of the disease, as there might be a possibility that they were latent in the plant during the earlier stages of the disease and only developed superficially during the later stages.

According to Jenkins and others, these rusted spots which

Jenkins, E. H. Studies on the Tobacco Crop of Conn. Bul. 180, p. 56 (1914).

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are sometimes observed are caused by a drying out and disintegration of the cell tissue which has been weakened by the disease, and which thus forms a suitable medium under favorable conditions for the development of secondary fungi and micro-organisms.. This view is also held by the writer as a result of observation extending over a series of years.

In not one instance has it been possible to obtain fungi from plants affected by the mosaic disease which when ~~re-inoc-~~ used to inoculate healthy plants ulated under varying conditions, was not capable of reproducing the mosaic disease.

MICRO-ORGANISMS AND THE MOSAIC DISEASE.

Among the many theories advanced regarding the cause of the mosaic the chief one for some time, particularly among the earlier investigators, was that of bacterial infection either through the agency of infected soil or otherwise. Mayer,

Mayer, A. Over de in Nederland dikwijk voorkomende Mozaikziekte der Tabak. Land. Tijdschr. (1885).

in his rather extended study of the disease, came to the conclusion that it was caused by bacteria, but was unable to isolate the organism. Prilleux and Delacroix claimed to have found an

Prillieux, E. E. and Delacroix, G. Maladies bacillaires de divers negetaux. Compt. Rend. Acad. Sci. Paris 118:668-671 (1894).

organism associated with the mosaiced leaves, but their descriptions leave one in doubt as to whether they were working with the true mosaic disease or not. It is very probable that they were dealing with another disease which occurs in France but which is somewhat different from the mosaic disease. The next important work on the bacteria supposedly connected with this disease was done by Iwanowski. He isolated several organisms

Iwanowski, D. Uber die Mosaikkrankheit der Tabakspflanze, Zeit. f. Pflanzenkrank 13:1-41, pl. 1-3 (1903).

from the juice of diseased leaves, and by re-inoculation was able to cause infection, but only in a very small number of instances. This he explains by a probable attenuation of the organism when grown on artificial media. Hunger, in a very

Hunger, F. W. T. Untersuchungen und Betrachtungen uber die Mosaikkrankheit der Tabakspflanze. Zeit. f. Pflanzenkrank 15:257-311 (1905).

critical review of the bacterial theory stated that he was unable in any way to substantiate the findings of Iwanowski, and that although he observed certain bodies in the cells he was not able to classify them as either bacteria or plasmodia, as they disappeared after heating with phenol chloral hydrate while the rest of the cell contents were unaffected. More recently Allard

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Allard, H. A. Mosaic Disease of Tobacco. U. S. D. A. Bur. Plant Industry, Bul. 40 (1914).

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has advanced the opinion as a result of his investigations that the disease is parasitic in nature, but does not attempt to discuss the character of the parasite, and apparently has made little attempt to demonstrate anatomically the presence or absence of bacteria. Hunger's work is probably the most satisfactory of its kind along this line.

The writer has made examinations of diseased plants, sectioning leaves, stem and even the roots, but has never been able satisfactorily to demonstrate the presence of bacteria in the tissues. In this work a variety of stains were used, chief of which, however, were Ziehl's carbol fuchsin and Heidenhain's iron haemotoxylin.

It is to be noted in this connection that all investigators have apparently confined their studies to the leaves or part of the plant in which the disease showed itself, and very few attempts if any have been made to study the question of the possible presence of bacteria in tissue removed from the diseased portions . In view of the fact that the juice from

all parts of a diseased plant is infective it would be natural to suppose that if bacteria were the causal agent, it should be possible to demonstrate their presence in the different parts of a diseased plant.

This has never been done, and in the writer's study of the anatomy of diseased plants it has never been possible to demonstrate the presence of bacteria in the different tissues.

In the light of these later investigations the evidence points overwhelmingly to the absence of bacteria in the present day sense of the term, as the causal agent of the disease.

INSECTS AS AGENTS IN THE DISSEMINATION OF THE MOSAIC DISEASE.

The fact that many fungous and bacterial diseases are often transmitted by insects has been long known and thoroughly established, but until Allard (l.c.) called attention to the fact that the mosaic disease could be carried by aphids, and one in particular (Macrosiphum tabaci Perg.) nothing had been published on this phase of the matter. Allard in well controlled experiments demonstrated beyond a reasonable doubt that the disease was so communicated. Clinton (l.c.) made a few observations on the infection of healthy plants by the tobacco horn worms which had been feeding on diseased leaves, but was unable to demonstrate that the disease could be so transmitted either by the excretor ejected or by its biting and feeding on the healthy plants. His results were negative in the few experiments made.

Observations made in the field during the progress of the

writer's work have shown that the disease can be communicated by biting insects such as the tobacco horn worm, grasshoppers and a small black flea beetle of more or less common occurrence in our fields.

Occasionally aphids have been found infesting the leaves of tobacco in our fields, but so far as could be judged were present in too small numbers to be active agents in transmitting the trouble. As a rule comparatively few aphid infestations are found in our tobacco fields.

In the greenhouse during several winters tobacco plants grown in benches were infested with white fly ,and it was at first feared that they might carry the infection from diseased to healthy plants in the same benches. This, however, was not the case and it has never been possible to demonstrate positively that the white fly is an active agent in the spread of the disease. This insect is of course of rare occurrence in our fields, but may possibly do damage south. It apparently feeds and breeds freely under greenhouse conditions on the underside of the leaves.

In order to ascertain more definitely as to the possibility of infection by these insects, adult white flies from badly mosaiced leaves were carefully removed and placed on the underside of the leaves of tobacco plants enclosed in a small cloth covered cage, and were allowed to remain on the tobacco leaves of the plants in these cages for four days. After this length of time the plants were removed from the cages and placed on the bench at some distance from the original benches. On

none of the plants did mosaic develop. The plants were later taken and placed in close juxtaposition to those in the original benches, which were at this time heavily infested with the white fly, but although the plants were left to maturity, no cases of mosaic developed on them. The writer's observations on the activities of aphids as carriers of infection have not been so extensive as in the case of the white fly, as only minor infestations of the former occurred in the greenhouses and the indications pointed to the fact that although there were a certain number of aphids present on the leaves of both healthy and diseased plants, so far as it was observed no cases of infestation from this source arose, as the mosaic did not develop except on an average of one case out of thirty except on the plants which were inoculated with the juice from diseased leaves. It should be stated, however, that aphids present in the greenhouse were not of the same species as that under consideration by Allard. There is no reason to doubt the accuracy of his observations on this subject so far as the writer is able to judge from a perusal of his work.

The question of insects as carriers of the mosaic disease as well as other diseases is still open to discussion, and it may be that in the case of the mosaic that a very heavy infestation of aphids is necessary to bring about a successful infection of healthy plants, as the amount of active infective material carried by insects would in any case be very small, and accumulative effects of the action of several insects might be necessary to introduce about a sufficient injection to transmit the disease. A discussion of the probable effects of insect

infestations due to their being carriers of bacteria or active infective material of another character will be taken up later in this paper.

ENZYME ACTIVITIES IN HEALTHY AND MOSAIC DISEASED TOBACCO.

The more recent investigators of this peculiar trouble have come to the conclusion that it is either caused by ultra-microscopic organisms, or is the result of malnutrition in some form which so affects the activities of some of the enzymes, that they, or products of their activities, are able to induce the trouble ~~by inoculation~~, even when they are present in a very extreme dilution. This latter view is of course vigorously opposed by those who believe that all diseases which are infectious must possess a parasitic etiology. In the opinion of the writer, however, until more is known about the so-called "viruses" and ultra-microscopic organisms, the declaration that the "enzymatic" theory of the cause of certain diseases of plants is absurd is open to question, as in no case, so far as the writer is aware, has the presence of ultra-microscopic organisms been demonstrated in the tissues of plants.

The study of enzymes in relation to diseases, particularly those of a so-called physiological nature , has not been extensively gone into as yet by investigators, but it is believed that a study of their activities and reactions should be made not only in the case of the physiological troubles but also those caused by fungi, as it is the writer's firm belief that the activities of a greater number of the fungi and their effects

on the respective hosts are in a great measure due to the action of either exo- or endo- enzymes of the fungi concerned; that there is a possibility that the future may show a great advance in the study of host resistance, when the conditions under which enzyme activity in fungi and bacteria takes place are better known, and plants may possibly be bred to a condition of producing either a medium in which these activities cannot take place or will produce anti-enzymes which will prevent the activities of the enzymes contained by the respective fungi.

Review of Previous Investigations.

Although many have made a study of this disease very few have concerned themselves with the question of the enzyme activities; among the first to make mention of this phase of the question was Woods (l.c.) who found that the enzymes designated as peroxidases were at least diffusable and occurred apparently in larger amount in diseased leaves than in healthy ones, also that their action was twice as strong in the light green areas as in the darker portions of the leaf. Koning (l.c.), as a result of his investigations, came to the conclusion that the disease was caused by a certain enzyme, which he stated to be oxidase, and the action of which he described. He believed that it was formed in the plant under certain conditions. Heintzel also found oxidizing enzymes present which were more active,

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Heinzel, K. Contagiose Pflanzenkrankheiten ohne Microben, mit besonderer Berucksichtigung der Mosaikkrankheit der Tabaksblatter. Erlangen, 46 p., 1 pl. (inaugural Dissertation). (1900).

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if not present in greater amounts in diseased plants than in the normal plants. Woods later (1902) in his work on the mosaic disease verified his former observation and stated further that the diastase activity was much inhibited in the case of diseased plants. He attributed the lessened diastase activity to the presence of excessive amounts of oxidizing enzymes, and showed experimentally that diastase action is inhibited by the presence of oxidizing enzymes. This is the only work that has been accomplished up to the present time, in so far as relates to a study of the enzyme activities involved. Only two enzymes have been condidered, namely, the oxidase and diastase, and it should be stated that in light of later developments in the determination and estimation of enzyme preparations and activities these results might well be open to some criticism.

Loew while working with tobacco discovered the presence

Loew, O. Catalase A New Enzyme of General Occurrence, with Special Reference to the Tobacco Plant. U. S. D. A. Bur. Plant Industry. Bul. 68 (1901).

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of an enzyme which he called catalase, but he made no observations relative to its activities in the case of diseased plants.

Plan and Methods of Investigation.

In the experiments detailed below the enzymes under discussion were studied (1) with regard to their presence or absence in (a) leaves, (b) stems and (c) roots of healthy and diseased plants. This was considered necessary as it has been found that irrespective of the parts showing visible symptoms of the disease,

the juice from all other parts also is capable of transmitting the trouble. (2) with regard to the age of the plant: (3) with regard to the growth of the plant under different conditions. These will be discussed in detail under their respective sections.

The methods employed for the estimation were for the most part those which by experience have been found satisfactory and in the main give quantitative results; in some cases the results are more or less qualitative in nature, owing to our present insufficient knowledge of the methods of isolation and action of the enzyme involved.

It should be stated that the plants used in the experiments were both field and greenhouse grown, but no essential differences in results were obtained from the two series. The individual experiments will not be given in detail, but as the determinations of any given series were made in every case in the same manner, only average results with the maximum and minimum readings will be given. The experiments are, however, described in sufficient detail to enable those interested to follow the methods employed closely enough to check up the work of the writer.

Catalase.

Leaves. A comparison was made of the catalase activity of healthy and diseased leaves, as it had been noted as early as 1908 that there was apparently a great difference between the catalase activity of healthy and mosaic diseased tomato leaves, and the same was found to be true in the case of tobacco. At that time only rough determinations were made, but since then

The writer has made hundreds of determinations, the results of which have borne out the observations made then, and indisputedly established the fact that there is a wide difference in the catalase activity of healthy and diseased leaves.

In all the experiments freshly collected material was used, and the determinations made almost immediately after collection. The usual procedure was as follows:

A weighed amount of leaf was ground thoroughly with a weighed amount of acid washed sand and a certain volume of double distilled water, and the whole washed into the apparatus with sufficient double distilled water to bring the volume up to the standard volume used in the particular series in question. This, of course, gave to each flask a standard constant dilution value. To this mixture was then added a like volume of 1 % solution of Merck's Perhydrol, thus making the H_2O_2 concentration of the total mixture 0.5%. The amount of oxygen liberated in ten minutes was arbitrarily taken as the measure of enzyme activity. Several different forms of apparatus were used, but for large amounts of leaf the ordinary water displacement method was found to be very satisfactory. In making determinations where the amount of material was very small the apparatus designed by Löhnis was found to be more convenient. Practically all determinations were made at temperatures ranging from 17° - 23° C. The action of the catalase is much accelerated by shaking, as pointed out by Loew, and each test was shaken under exactly similar conditions in all the determinations made. It was found necessary to use this method for the determination

of the catalase activity, as any method involving titration, such as the permanganate method, was unsatisfactory, due to the action of certain constituents of the tissue on the reagents.

Table I shows the relative amounts of oxygen developed in normal tobacco leaves, and it is to be noted that the catalase of the dark green leaves was much more active than that of the light green leaves. This was found to hold true to a certain extent for light and dark green leaves even on the same plant. The basal leaves of older plants, which in some cases were almost mature, and of a lighter color than the middle and upper leaves, developed in every case relatively less oxygen. This was particularly true in the case of Havanna tobacco. Broadleaf did not show such a wide divergence, but it should also be stated that in the Broadleaf plants employed in the determinations the basal leaves did not show any great color differences.

As will be noted some of these experiments were made with plants grown under field conditions, but a greater number were made with plants grown in the greenhouse, under control conditions.

(Table I here)

These results show that the catalase activity varies somewhat even in healthy plants, dependent upon age and also apparently on the general condition of the plant. It shows clearly also that in plants of approximately the same age the catalase activity varies somewhat between plants with dark green leaves and those with light green leaves.

Even on the same plant this holds true as can be seen from the results tabulated below.

(Table II here)

An examination and determination of the catalase activity in diseased leaves shows that the amount of oxygen developed is relatively much less than in the case of healthy leaves. In the table below are given some of the results obtained from diseased leaves. In these experiments the leaf tissue was used without reference to the light and dark areas of the individual leaf. It is significant that the activity is very much less than in healthy leaves. All the plant used in this series were badly diseased. It should be stated that in apparently mild cases of the disease the variation from the normal catalase content was not so great. The results shown here can hardly be compared with those given in Table I, as the plants were not in some cases of the same age, nor were they grown at the same time.

(Table III here)

In the next table will be found the comparison of the results of catalase activity from healthy and diseased leaves from plants grown at the same time and under identical conditions. The plants were inoculated artificially in as uniform a manner as possible.

(Table IV here)

The values here obtained simply substantiate those given in preceeding tables but in addition allow of a direct comparison.

As has been previously stated the leaf tissue was used in the preceding experiments without regard to the light and

dark green patches on the individual leaf.

It was thought that an examination of the light and dark green areas of individual leaves of mosaiced plants might give a clue as to whether the activities of the catalase were inhibited in one or both of these areas in comparison with a leaf from a healthy plant of approximately the same age and color.

It was found that the catalase activity of the dark green areas approached that of the normal leaf of the same color while the catalase activity of the light green areas was much below normal, even in the case of a light green normal leaf being used for comparison. The values obtained are given in Table V.

(Table V here)

Diastase.

It is a well known fact that diastase is intimately connected with metabolism in the leaf in practically all chlorophyll bearing plants, as well as in many of the fungi, and the relations of the activities of diastase in the mosaic disease are of rather significant importance, as can be easily shown. It was pointed out several years ago by Woods (l.c.) that the action of oxidizing enzymes when present in solutions containing diastase tended greatly under ordinary conditions to inhibit the activities of the diastase. Turning more particularly to the mosaic disease, he made the observation that in the cells of the light green areas, although they formed starch practically in a normal manner so far as could be observed, the

starch was not translocated, and that in the morning there was practically as much starch present as at night, which is not the case in a normally functioning leaf. In this case it was found that practically all the starch disappeared in the night and was translocated.

Recently there has been more or less contention as to the exact method of action of diastase on starch, and within the last two or three years important investigations have resulted in the opinion, substantiated more or less in detail by the results of certain investigations that the diastase of the older writers is not one enzyme alone, but is made up of at least two components. The first of these breaks down the starches into, or as far as, the erythro- and achro-dextrine stage; the second component taking up the action from this point and completely hydrolizing the starch to the sugar compounds which are found to be present as the next step in the process of metabolism.

It was in the light of these investigations that the writer took up the question of the diastase activity in the mosaic disease, and it was found to be less active in the leaves which showed severe symptoms of the disease, than in those which showed only a slight trace. There was, however, apparently a greater or less breaking down of the starch in all the leaves examined so far as could be determined by the colorimetric methods, which, although not altogether satisfactory, may be relied upon as much as any of the present known methods of determinations. The starch did not take on the color of the normal starch in the healthy leaves, varying from the yellow-

brown to a reddish or violet coloration, dependent on the strength of the indicator used . The strength of the iodine solution used in this case was a 50th normal iodine potassium iodide solution. This would show that the starch to a certain extent had been acted upon at least partially, by the diastatic ferments, and would indicate also that it was the first of the components above mentioned which was more active, and the second was more or less inhibited in its action. In the normal leaf, of course, there was a certain amount of starch present indicated by the blue coloration of the granules. It was slight, however, compared to that in the diseased leaves, and in no case was there any of the brown color, complete hydrolysis having apparently taken place very rapidly. This would indicate that the oxydizing enzymes, of which we have made mention and which are present in excessively large amounts in the diseased areas of the leaf, do play an important role in the controlling or inhibiting of the activities of the diastatic ferments, but not on the diastase in the old conception of the word. Rather it might be said the action is on the primary enzyme concerned in the diastatic activity, as, if the opinion above advanced is true, as it would seem to be from the unpublished investigations of Roessler, of the University of Prague, who was able to separate by salting out from a very carefully prepared solution of the ordinary diastase at least two components having the action above mentioned. In no case, as indicated by the color reaction obtained, did we get a complete inversion of the large amount of starch, the process only being carried on, as has been indicated, as far as the erythro- and achro-dextrine stage. It was attempted in our experiments to

isolate or rather separate out diastase in a more or less pure form from the leaves of healthy and diseased plants, and although certain results were obtained, it was rather a difficult matter, as in the writer's experience it has been found that diastase is one of the most difficult of the enzymes to purify to any extent. The protective colloids, etc., during the purification are separated away from the enzyme aggregate and the purer ferment becomes less active. The reason for this cannot be very well explained at the present, but it is the experience of all investigators with diastase that this is a fact. However, results were obtained which seemed to indicate that the diseased leaves contained relatively less diastase than do the normal healthy leaves.

Chlorophyllase.

This enzyme has been found to be always present with chlorophyll, in amounts directly proportional to the amount of chlorophyll present, and according to Willstätter and Stoll

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Willstätter and Stoll. Unt. uber Chlorophyll VI und VIII. Uber Chlorophyllase. Liebig's Ann. der Chemie. 378, 18 (1910); 380, 148 (1911).

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does not bring about an hydrolysis but an "alcoholysis"

$RCOOC_{20}H_{39} + C_2H_5OH \rightleftharpoons RCOOC_2H_5 + C_{20}H_{39}OH$

in the presence of ethyl alcohol. It forms the alcohol phytol $C_{20}H_{39}OH$ from the radical in the presence of ethyl alcohol and not water only.

Very little is known about its action in the plant cell and although the writer was able to demonstrate its presence in both healthy and diseased leaves, no quantitative data were

TABLE I.

Catalase Activity in Healthy Leaves.

Wt. leaf used = 3 grams
Time of action = 10 min.
Temperature = 17 - 23° C.

Vol. of leaf + H_2O = 100 c.c.
Vol. 1% H_2O_2 added = 100 c.c.
g = greenhouse. f = field .

Series	Variety	Amount of O. Developed Max.	Min.	Ave.	Color of Leaf	Number of Determinations	Age of Plant
A	Havana (g)	139 c.c.	97 c.c.	119.8 c.c.	Dark	40	Half grown
B	"	103 c.c.	48 c.c.	56 c.c.	Light (basal)	26	Nearly mature
C	"	94 c.c.	61.5c.c.	77.5 c.c.	Light (whole plant light)	7	Half grown
D	Broadleaf (g)	126.4 c.c.	101 c.c.	113.7 c.c.	Dark	3	Half grown
E	"	134 c.c.	119.5 c.c.	126.3 c.c.	Dark	11	Nearly mature
F	"	106.7c.c.	78.2 c.c.	93.4 c.c.	Light (basal leaves)	5	Nearly mature
G	Havana (f)	176 c.c.	115.5 c.c.	124.8 c.c.	Dark	19	Half grown
H	"	147.6 c.c.	93.1 c.c.	100.2 c.c.	Dark	14	Nearly mature
I	"	121 c.c.	72.9 c.c.	91 c.c.	Light	6	Half grown

TABLE II.

Catalase Activity of Light and Dark Leaves

From Same Plant.

(Plants nearly mature) (Proceedure as in Table I)

Plant No.	Number of Determinations	Light Leaves C.C. of O. Developed (Ave.)	Dark Leaves C.C. of O. Developed (Ave.)
B_1	4	51.8 c.c.	119.8 c.c.
X_{11}	3	62 c.c.	125.5 c.c.
104	3	71.4 c.c.	93.7 c.c.
A_{17}	6	58.1 c.c.	79.3 c.c.

TABLE III.

Catalase Activity in Diseased Leaves.

(Plants badly diseased.) (Proceedure as in Table I)

Plant No.	Number of Determinations	C. C. O. Developed (Ave.)
P_6	8	47.2
R	6	32.8
3_a	9	54.5
Ax	11	69,6
Total	34	51.0 c.c.

TABLE IV.

Catalase Activity in Leaves of Healthy and Diseased Plants of Same Age.

(Proceedure as in Table I)

Leaves	Number of Determinations	C.C. O. Developed (Ave.)
Diseased	10	52.3
Healthy	10	119.0

TABLE V.

Catalase Activity in Diseased Leaves.

(Comparison of light and dark green areas.)

(Proceedure as in Table I)

Series	Number of Determinations	C.C. of O. Developed (Ave.)	
		Light	Dark
X	4	42.1	73.6
0	3	37	95.4
21	8	54.3	103.0

secured as to its relative abundance in healthy and diseased tissue. Until better methods are worked for its purification and rapid determination it would be futile to hazard an opinion in regard to its specific action in the cell.

Oxidases and Peroxidases.

Woods (l.c.) was one of the first to observe that in mosaic diseased leaves the oxidase activity was greatly increased. Since then it has been found that in the curly dwarf disease of the potato and sugar beet the oxidase activity is greatly increased in the diseased leaves as compared with that of the normal. These two diseases have been for the most part regarded as physiological, and it is a significant fact that this excessive activity of oxidizing enzymes has been more frequently noted in diseased of this character, than in those which are caused by bacteria or fungi. The reaction of the host is apparently different.

Bunzel has noted that the oxidase activity varies with

Bunzel, H. H. Oxidases in Healthy and Curly Dwarf Potatoes. Jour. Agr. Research, Vol. II, 5:373-404 (1914)

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the age of the plant in the curly dwarf disease of potato, reading its greatest activity when the plant growth ceases.

The writer has also found this to be true for tobacco to a certain extent, and always met with greater activities of the oxidases as the leaves were approaching maturity. This was very marked in the case of normal plants, but not so much in the case of diseased leaves.

In the writer's examinations of healthy and diseased tissue not only qualitative colorimetric methods were employed but also Bunzel's oxidase apparatus was made use of. This has been found to be the most satisfactory method for the quantitative estimation of oxidase activity.

Bunzel, H. H. The Measurement of the Oxidase Content of Plant Juices. U.S.D.A. Bur. Plant Ind., Bul. 238 (1912).

A few of the quantitative results obtained are given in Table VI.

(Table VI here)

For the qualitative determinations the usual guaiac test was employed. The guaiac test for oxidases and peroxidases is too well known to require an extended explanation. The results obtained by this method in every case showed the diseased leaves to contain much more oxidases than the healthy ones of the same age; this was also true for peroxidases but here, of course, the reaction with guaiac was somewhat masked owing to the presence of the oxidases and their reaction.

In the examinations of the roots of healthy and diseased plants the same condition was observable; there was always an excessive activity of the oxidixing enzyme to be noted.

In going over the results of the experiments with the enzymes in question, the main point brought to the attention is that there is in all diseased plants an excessive activity of the oxidizing enzymes and a corresponding decrease in the activity of the diastatic enzymes and datalase. This at least

indicates a very much disturbed equilibrium and a consequent derangement of normal function on the part of the cells. Naturally the ones most affected by this disturbance are the dividing or meristematic cells, as these are the cells upon which the plant is dependent for its subsequent growth, and any deviation from the normal is more likely to be indicated in the development of these cells than in those of the other parts of the plant. Any change in function induced here will leave its imprint to a greater or less extent on the cell during its subsequent existance, hence the peculiar manifestations of the disease in the leaves.

It is true that plants attacked by parasites sometimes show an excessive activity of certain enzymes, but, as a rule, the disturbance is more local in its nature. It is also a fact that malnutrition, such as partial starvation, drought, etc., will bring about an excessive production or activity of the oxidizing enzymes in particular, as has been pointed out by Bunzel, of general distribution throughout the plant, but this is purely physiological in nature.

EFFECT OF COLORED LIGHT ON THE MOSAIC DISEASE OF TOBACCO.

In connection with work on the mosaic disease of tobacco it had long been notedin that section of the Connecticut Valley where the crop was grown under shade, that the plants appeared to be much less affected with the mosaic disease than were those grown in the open. This fact has already been noted by Sturgis

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Sturgis, W. C. On the Effects on Tobacco of Shading and the Application of Lime. Conn. Agr. Exp. Sta. Ann. Rept. 23:252-261 (1899).

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in Connecticut. Investigations were outlined in conjunction with the other work on this disease already under way, relative to a study of the effects of various light conditions on the intensification or reduction of the disease. While the writer's preliminary work was in progress Lodewijks published a paper

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Lodewijks, J. A. Jr. Zur Mosaikkrankheit des Tabaks. Rec. Trav. Neerlandais, Vol.7, 107-129 (1910).

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on the effects of colored light on mosaic diseased plants, and as a result of his experiments stated that a cure was effected by blue light; red light diminished the disease and suffused light checked it somewhat. In brief, his methods of experimentation and conclusions were as follows:

The diseased leaves of a plant were covered with a cloth hood of the desired color, of a sufficient size to allow ample

room for growth. The apparently healthy basal leaves were left uncovered and exposed to the normal daylight. After a time the hoods were removed and it was found that in the case of the plants exposed under the blue hood, a cure was effected; those exposed under a red hood showed a diminution in the severity of the disease and in the plants exposed to the suffused light the disease was somewhat checked. The cloth used for the red and blue hoods was a rather coarse cotton material similar to that used for making flags.

Many investigators had noted the apparent beneficial effects resulting from growing diseased plants in suffused light, but Lodewijks was the first to study the effects produced by colored light, although Baur appears to have made some observations on this point. As in no case could the writer find that Lodewijks in his work had reinoculated from the apparently cured plants to healthy ones, to prove the presence or absence of the infective agent ~~(?)~~ and as it is often present and active in apparently healthy leaves of diseased plants, as has been shown many times, it was thought necessary to settle the point as to the presence or absence of the agent in plants treated as in Lodewijks' work.

DESCRIPTION OF EXPERIMENTAL METHODS.

The method of treatment of diseased plants was in every way similar to that employed by Lodewijks as to texture of cloth, methods of covering the plants, etc. The cloth covers were held away from the plant by means of wire hoops, and the

cloth was tied around the stem of the plant below the diseased leaves. Plate I shows a hood in place over a field grown plant, and gives a clear idea of the arrangement of the hoops, etc.

The cloth used was a coarse grade of cotton and the colors were cadmium orange, oxe-blood red and indulin blue.

Ridgway, Robert. Color Standards and Color Nomenclature. Washington, D. C. (1912).

Plants showing well developed symptoms of the mosaic disease were selected for the experiment, none of which had less than four characteristically diseased leaves, the lower remaining leaves apparently healthy. The hoods were placed over the diseased leaves as above noted and left on for the required time, in most of the experiments twenty to thirty days. At the end of this period the hoods were removed and the plants carefully examined for visible symptoms of the disease. Two leaves from the upper, i.e., the part under the hood, portion of the plant were removed under absolutely aseptic conditions, the juice expressed and healthy plants inoculated with it by means of glass capillaries inserted just below the terminal leaflets. Control inoculations with distilled water and boiled juice were also made at the same time. The plants after the removal of the leaves above mentioned were allowed to grow to maturity under normal light conditions.

Most of the experiments were carried on in the greenhouse, where temperature and other conditions were under more direct control than in the field; although field experiments later

repeated gave the same results, but, of course, in this case there was a greater chance of subsequent infection through careless handling, insect attacks, etc. In the following paragraphs there has been tabulated the results of a typical series of experiments relative to the effects of light on mosaiced plants.

Experimental Data.

Red Cloth. Three plants were covered with the red cloth hoods for twenty days. The covers were then removed and in all cases visible symptoms of the disease were still present, although the color variation between light and dark green areas was not so marked as at the beginning of the experiment. All the new growth, in addition to the leaves diseased at the time the hoods were put on, also showed the mottling distinctly. A week after the hoods were removed all the plants still showed the disease in undiminished severity.

Healthy plants inoculated with the juice from the leaves confined under the hood became diseased in from nine to eighteen days time. Controls inoculated in the same manner with boiled juice from the same leaves and with distilled sterile water remained with very few exceptions healthy. Table I gives the results of the inoculation experiments in one series.

Table I.

(next page)

Table I.

Results of Inoculations with Juice from Plants Grown under Red Hoods.

Plant no.	No. of healthy plants inoculated with juice from leaves of treated plant.	No. of inoculated plants showing mosaic at end of 18 days.
All	6	6
B 1	7	6
C 1	4	4

Controls inoculated with boiled juice, 10; diseased in eighteen days, 1.

Controls inoculated with distilled, sterile water, 10; diseased in eighteed days, 0.

From the above results it may be seen that there was a diminution in the color variation in diseased leaves; it was not of a permanent character, the plants all showing the disease in undiminished severity after a short exposure to normal daylight. The active principle of the disease was still highly infectious.

In a second series the hoods were allowed to remain over the plants for thirty days, as it was thought that a twenty day exposure might have been too short, but no appreciable variation in the results was obtained as a result of the longer treatment.

Orange cloth. Instead of using a hood for testing the effects of suffused light, an orange colored hood was used, and in this series two plants were covered with orange hoods for a

period of thirty days. On removing the covers it was found that the visible symptome of the disease were if anything, intensified. The growth was somewhat more spindling, the leaves narrower, and the light and dark green areas very clearly defined. Infection was produced from both plants by inoculation into healthy plants. The infective agent was very active and highly infectious.

Blue cloth. The diseased parts of three plants were covered with blue cloth hoods as in the preceding experiments for a period of twenty-five days. The covers were then removed and a careful examination of the leaves made. On plants (A_2) and (B_2) no visible symptoms of the mosaic disease could be observed, although a slight tendency towards curling was noticeable on a few of the leaves. The leaves were all uniformly light green in color, and aside from this, appeared normal. Plant (C_2), however, showed on two leaves a slight mottling. Two weeks after the hoods were removed, plants (A_2) and (B_2) did not show any marked symptoms of the mosaic disease other than a faint mottling of a few leaves, not sufficient, however, to seriously injure the leaf. Plant (C_2) developed mosaic again in the same length of time, but not as seriously as before the treatment. It may be that the mottling on (A_2) and (B_2) was due to the maturing of the plant, although this motlling is usually distinctive enough to be readily differentiated from that caused by the mosaic disease.

Healthy plants inoculated with the juice of leaves from plants (A_2), (B_2) and (C_2) contracted the disease almost without exception. Controls inoculated with boiled juice failed to

develop the disease. Table II gives the results of the inoculations.

Table II.

Results of Inoculations with Juice Plants Grown under Blue Hoods.

Plant no.	No. of healthy plants inoculated with juice from leaves of treated plant.	No. of inoculated plants showing mosaic at end of 18 days.
A 2	8	5
B 2	4	7
C 2	10	9

Controls inoculated with boiled juice, 6; in eighteen days, 0.

Controls inoculated with distilled, sterile water, 6; diseased in eighteen days, 1.

The above results show that when blue light is used there is a suppression of leaf color variation more or less permanent in character, the treated plants, with one exception, showing no typical symptoms of the disease for at least two weeks subsequent to the removal of the hoods. It cannot be said, however, that the disease was controlled, as inoculation of healthy plants with the juice from these leaves produced the disease in nearly every case.

The infective agent of the disease was still very active in the apparently normal fully recovered leaves, and was highly infectious.

Discussion of Results.

The results of these experiments do not agree entirely with those obtained by Lodewijks, particularly in the case of action of the blue light, inasmuch as the plants covered with the blue hoods, although showing an apparent recovery from the mosaic, still contained the infective agent of the disease, and by inoculation with the juice expressed from these plants into healthy plants the disease was again produced in practically all cases. It should be noted that the visible symptoms of the disease were suppressed, the reason for which may be as Allard (l.c.) suggests in his work on the mosaic disease of tobacco. He states with respect to Lodewijks' observations,-- "If the malady in question was true infectious mosaic disease, one is inclined to believe that covering the young plants temporarily reduced the color contrasts of the mottled areas. These changes may have led Lodewijks to conclude that a partial or a complete cure had been effected in his experiments."

It might be inferred from the above that on the removal of the hoods, exposing the plants to normal daylight, they would soon regain the color contrast, but this is not entirely so in the case of the blue light, as has been shown. The apparent recovery, therefore, is not entirely the result of a suppression of color contrast due to the action of blue light on the leaves, as suggested by Allard, but is undoubtedly so in part.

It is evident that the treatment of plants as above recorded does not destroy the infective agent of the mosaic disease, whatever may be its character, the treated leaves apparently

still containing this active principle in a latent state, very probably in the same manner as do the parts of a plant which do not show visible symptoms of the disease, as the stem, lower leaves, roots, etc., the juice of which is often highly infectious. It would appear from the results that the new terminal growth subsequent to the removal of the hoods would develop the trouble and this was the case in plant (C_2) but not apparently so with plants (A_2) and (B_2). Lodewijks' opinion, therefore, that in the plant a "virus" and "anti-virus" are present and that certain abnormal conditions cause the "virus" to be produced in excess, bringing about a mosaiced appearance, while if the "anti-virus" is produced in excess, immunity is secured, will hardly hold, as it is clearly shown that the infective principle is present and active.

It is significant to note that under the influence of blue light both the assimilation and starch formation are decreased, thus bringing about a partial starvation, as it were; not, however, serious enough to reduce greatly the <u>total</u> starch formatbn and assimilation of the whole plant, while at the same time the chlorophyll production is very little changed if a comparison of the color of the normal and treated leaves can be taken as a basis of such a comparison. This latter fact has already been noted by Lodewijks in his work on the disease.

It is, therefore, indicated by the results obtained in the preceding experiments that the different colors have little or no effect on the infective agent of the disease, but in the case of the blue there is a strong depression of the macroscopic symptoms of the disease.

Summary.

Exposure of diseased portions of plants to red light for a period of thirty days has no effect on the infective agent of the mosaic disease, but the leaf color variation between healthy and diseased portions of a leaf are temporarily suppressed. On exposure to normal daylight, the diseased leaves rapidly assume their mosaiced appearance.

Orange light intensifies the disease; no suppressive effect being noted in any case.

Treatment with blue light causes an apparent suppression of the visible symptoms of the mosaic disease, more or less permanent in character, but the infective agent is still present in such leaves and is still highly infectious.

In none of the experiments with red, orange and blue light was there any marked diminution in the activity of the infective agent when inoculated into healthy plants.

PLATE

Showing Hood in Position over Plant.

MOSAIC DISEASES OF THE SAME CHARACTER AS THAT ON TOBACCO.

It has been proved that plants of the Solanaceae are especially susceptible to the true mosaic, and it has been possible to produce this typical mosaic disease in them by inoculations with the juice from diseased tobacco leaves in many cases. Thus, Clinton showed that the mosaic disease observed

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Clinton, G. P. Report of Conn. Agr. Exp. Station, Part XII:857-858 (1908).

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on tomato was apparently identical with that of the tobacco and was able to cross inoculate from tobacco to tomato and back again, producing in all cases a typical mosaic disease. Allard (l.c.) more recently has shown that other genera of the Solanaceae -- Datura, Solanum and Capsicum, Physalis, Petunia, Hyoscyamus, as well as Lycopersicon, are capable of infection from diseased tobacco leaves and contract the true infectious mosaic of tobacco. We find other apparently authentic reports of a mosaic disease occurring on the potato also. This so-called mosaic on the potato as reported by Orton and later by Melchers has been observed in a few instances by the writer, but while it exhibits apparently all the symptoms of the typical

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Melchers, L. E. Ohio Naturalist XIII, 8. The Mosaic Disease of Tomato and Related Plants, 149-175 (1913).

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disease, it has been impossible so far for anyone to reproduce the mosaic disease of tobacco on the potato by simple inoculation from tobacco, and so far as the writer is aware, no report has been made as to its infectious nature. There seems no doubt that we have on the potato a disease of the same general character as that on tobacco, but until careful investigations have shown that it is capable of transmission from diseased to healthy plants by inoculation, it should not be called a true mosaic disease. Indications point to its being of a slightly different character than the mosaic occurring on other Solanaceae. Schwarze reported a mosaic disease of the pepper exhibiting all the characteristics of the tobacco mosaic, and

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Schwarze, C. A. Relation of the Mosaic of Pepper and the Filiform Leaf of the Tomato to the Mosaic of the Tobacco. Abstract in Phytopathology 4:1:42 (1914).

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stated that small bits of diseased pepper inserted into healthy tobacco plants caused a development of true mosaic in the tobacco.

REACTION OF CAUSATIVE AGENT WITH VARIOUS SUBSTANCES.

We have seen that the enzymatic activities of the plant are very much disturbed in disease, also that it has been impossible to demonstrate the presence of any forms of bacteria or fungi either in the tissues themselves or in the expressed juice.

It is a fact as shown by practically all investigations that the disease is very infectious. This fact alone in the minds of many is sufficient to place the causative agent among the parasitic organisms. The field, however, is limited to that class of organisms designated as "ultra-microscopic" organisms, about which very little is known, and in the case of plant diseases, not even a semblance of the demonstration of the activities of such organisms has been made.

Owing to the fact that the enzyme activities are much changed as has been demonstrated in the preceeding pages, and also from the fact that not only the activities of the oxidizing enzymes were changed, but also the activities of others, it was believed by the writer with Woods and others, that the disease might be physiological in nature, particularly in so far as the causative agents not being a living organism in the ordinary conception of the word, was concerned.

So little is known about the action of the so-called ultra-microscopic organisms that it is an open question in the writer's mind whether this division should be the dumping ground for all parasitic diseases, about the etiology of which

little is known.

It is conceivable that other causes, not organic in nature, may be able to produce the manifestations of parasitism. Under this type of infection would be included infectious diseases caused by enzymes or the resultant product of the activities of a group of enzymes.

Certain reactions of the juice from diseased plants tend to confirm this view and in the following pages are given the results obtained by the writer and other investigators relating to the reactions of these juices with various reagents.

Drying: It has been shown by various investigators that the dried leaves of the mosaic diseased plants retain their infectious qualities for a long time. Beijerinck and Allard found that diseased leaves were capable of causing infection after being dried for periods of two years and eighteen months respectively. The writer has also used material three years old and obtained infection in a great majority of cases. The results obtained are given below.

Table VII.

Air dried mosaic leaves, finely ground and macerated with cold, distalled water. Leaves (herbarium specimens) three years old.

(next page)

No. of plants inoculated.	Point of inoculation.	No. of plants infected.	Per cent infection.
10	Below terminal leaflets.	10	100
12	Main stem near base	11	91
7	Mid-ribs of a basal leaf.	6	86
13	Mid-ribs of a basal leaf.	12	90

Filtration. Beijerinck (l.c.) has shown that the sap of diseased leaves still retained its infectious action when filtered through Chamberland filters, and Allard(l.c.) and Clinton (l.c.) have shown that it is still infectious when filtered through the Berkfeld filter.

These results have been substantiated by the writer. The percentage of infection, however, was somewhat lower than in the case of the Chamberland filter.

The average percentage of infection obtained in the writer's experiments was as follows:

Chamberland filter (average of 3 examinations) 91%

Berkfeld filter (average of 5 examinations) 63%

Resistance to antiseptics: The results obtained with various antiseptics such as are commonly used to prevent bacterial action are as follows:

(next page)

Table IX.

Antiseptic.	Amount of sap used.	Period of treatment.	Infection.
Toluol (2c.c.)	10 c.c.	2 months	(++)
Toluol (2 c.c.)	10 c.c.	4 "	(++)
Chloroform (sat.)	10 c.c.	2 "	(++)
Chloroform (in excess)	10 c.c.	2 "	(-)
Chloroform (sat.)	10 c.c.	4 "	(+)
Chloroform (in excess)	10 c.c.	3 days	(-)
Thymol (2%)	10 c.c.	2 months	(+)
Thymol (2%)	10 c.c.	4 "	(+)
Ether (2 c.c.)	10 c.c.	2 "	(+)
Ether (sat.)	10 c.c.	4 "	(+)
Formaldehyde (4%)	10 c.c.	2 "	(-)
Formaldehyde (4%)	10 c.c.	10 days	(+-)
Carbolic Acid (5%)	10 c.c.	2 months	(-)
Chloralhydrate ($\frac{1}{2}$ mol.)	10 c.c.	2 "	(-)
Chloralhydrate ($\frac{1}{2}$ mol.)	10 c.c.	20 hours	(-)

++ = very infectious.
+ = infectious (over 40%).
-+ = one or two cases of infection, possibly accidental.
- = no infection.

From the preceding table it may be seen that the sap containing the active principle of the disease varies greatly in its reaction to so-called antiseptics and other compounds. The writer has already pointed out in a previous publication that the

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Chapman, George H. The Influence of Certain Capillary-Active Substances on Enzyme Activity. Internat. Zeitschrift für physik.-chem. Biologie., IBand, 5u. 6 Heft. (1914)

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influence of certain capillary active substances on enzymes is very variable, aside from the specific toxic qualities of certain of these substances. In comparing the reaction of the sap containing the active principle to certain of these compounds we find that there is a similarity of reaction to that shown by the enzymes. In the paper above cited it was shown that those compounds which had an active effect on the surface tension had, as a rule, dependent on their physical properties (hydrocolloidal or lipocolloidal) a certain definite effect on enzyme activities.

Taking up the discussion of the results in detail we find toluol a compound which is not soluble in water to any great extent and, hence, behaving like a lipocolloid, having no effect on the action of the active principle contained in the sap. Toluol, as a rule, has a more or less definite inhibitory action on living organisms.

Chloroform, when present in the sap not to exceed saturation, behaves also like a lipocolloid as it is only very slightly soluble in the water, and we find in this case that the activity of the agent is not destroyed. Chloroform in excess, however,

does destroy apparently the active principle of the disease. It is noteworthy that this action of chloroform exactly parallels that found to be the case with enzymes.

Thymol, when used in 2 per cent concentration, is very often used as a preventative to bacterial action and also prevents the growth of fungi. We find, however, that when it is present in concentration not exceeding 2 per cent in the sap that the active principle still possesses its infectious qualities for some time.

Ether is a substance which, like chloroform, has a lipoid character, but which has a definite action on the surface tension lowering it considerably. Sap containing ether to the saturation point, which lowers the surface tension from 1.0 to about 0.619 was still infectious four months after treatment, although the percentage of infection was much decreased.

Formaldehyde. A solution of the sap containing approximately 4 per cent of formaldehyde was very injurious and at the end of two months no infection was obtained. At the end of ten days, however, plants were inoculated and two cases of mosaic disease were obtained from a series of eight plants, but it is believed that this may possibly have been an accidental infection. In formaldehyde, however, you have a compound which has a specific narcotic action on certain enzymes aside from its surface activities.

Carbolic acid. Where carbolic acid was added to a solution of the sap the active principle was apparently destroyed.

Chloralhydrate. In chloralhydrate we have a substance very soluble in water but not possessing any relatively great

surface activity. It has, however, a specific toxic action on the active principle of the disease and even after twenty hours no infection was obtained. These results with chloralhydrate are in complete accord with those obtained in the enzyme work previously mentioned.

Most of the substances used in the above experiments possess a very definite toxic action to all organisms, particularly bacteria and fungi. As to their effect on the so-called ultro-microscopic organisms the writer is unable to state, not having had the opportunity of working with so-called cultures of these organisms. The parallelisms between the surface tension effects of these substances on enzymes and on the sap containing the active principle of the mosaic disease is very striking.

Having shown that the causal agent is not bacterial or fungous in character we must eliminate for the present the supposition of the presence of a toxin or virus in the pathologist's conception of these terms, as it is usual to conceive of these substances as being either the product of the organism or the activity manifested by the organism itself. As to the production of toxins and viruses by the so-called ultra-microscopic organisms little is known. Noguchi was the first to apparently demonstrate that such organisms do exist and was able to cultivate an organism obtained from the brain of patients suffering from infantile paralysis. However, these organisms were always mixed with certain bodies probably of a protein nature and Noguchi, himself, so far has been unable to state absolutely which may be the active agent, although he naturally infers from his inocu-

lation experiments that the organisms found must be the causative agent owing to the extreme infectious quality of the disease. He, however, states, himself, that it is not absolutely clear to him whether the organism alone or a combination of this organism with the bodies found in culture associated with it are capable of producing infection. He does state, however, that in the case of <u>animal</u> pathology no such a symbiotic relationship has so far been observed. From the character of his statement, however, it is clearly indicated that he does not preclude the possibility of such a condition arising.

Adsorbents. It is a well known fact that kaolin, charcoal, etc., are very active agents sometimes, adsorbing enzymes and also many other substances such as proteins. In such a mixture as the cell sap there are present not only the enzymes, but also other compounds, and little was attempted in the way of experiments on the power of these substances to adsorb the active principle of the disease. An indication as to the effect of some of these substances was obtained however, as the following table shows:

Table X.

Adsorbent	Infection	
	Filtrate	Residue
Kaolin	(+)	(+-)
Finely divided animal charcoal	(-+)	(+)

It can be seen that there is apparently an adsorption of the active principle, but the exact effects of this could not at the time be determined. It is hoped that further work may be done on this phase of the matter. With the use of additional adsorbents of this character and more carefully prepared sap extract there is no doubt but what interesting results will be obtained.

It should be stated also that when the juice is poured on agar plugs held in a test tube the active principle is found to be infectious at different depths. This the writer has demonstrated by cutting successive layers from such plugs and inoc-

ulating into healthy plants. The percentage of infection, however, is rather low and not constant. Layers taken deeper than two centimeters below the surface failed to produce infection in the writer's experiments.

CONCLUSIONS.

1. Mosaic disease is not caused by bacteria or fungi. It has never been possible to demonstrate the presence of pathogenic organisms in the tissue of any part of the plant.

2. The disease is highly infectious, particularly when inoculated into young plants.

3. All facts at hand point to the active principle of the disease as being of an enzymatic nature; or of the character of so-called ultra-microscopic organisms.

4. It is not caused by a toxin or a virus, using these terms in our ordinary conception of their limitations.

5. Until more is known about the action of the so-called ultra-microscopic organisms the disease cannot be placed under diseases ascribed to this class or organisms; also in the light of its character and reaction of the sap it should not be placed therein until more proof of the existence of such organisms is at hand.

6. Many of the reactions exhibited by the sap containing the active principle are of such a nature as to indicate that the active principle is either an enzyme, an aggregate of enzymes or the product of enzyme activities.

7. The enzyme activities of diseased leaves are greatly altered; far more so than is usually the case in plants which are attacked by pathogenic fungi or bacteria, where the greater part of the alteration is local.

8. As a result of the writer's experiments it is believed

that the disease is caused by a disturbance of the enzyme activities, due to malnutrition or disturbed metabolism and not by any parasite.

9. The pathogenicity of a disease is not necessarily a proof that it is of parasitic origin, as it is conceivable that the same conditions may exist relative to enzyme activities, although perhaps not to such a great extent. This, however, cannot be stated without reserve.